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Chemical multi-contamination drives benthic prokaryotic diversity in the anthropized Toulon Bay

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ABSTRACT

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4 Investigating the impact of human activities on marine coastal ecosystems remains difficult
5 because of the co-occurrence of numerous natural and human-induced gradients. Our aims
6 were (i) to evaluate the links between the chemical environment as a whole and microbial
7 diversity in the benthic compartment, and (ii) to compare the contributions of anthropogenic
8 and natural chemical gradients to microbial diversity shifts. We studied surface sediments
9 from 54 sampling sites in the semi-enclosed Toulon Bay (NW Mediterranean) exposed to
10 high anthropogenic pressure. Previously published chemical data were completed by new
11 measurements, resulting in an in depth geochemical characterization by 29 representative
12 environmental variables. Bacterial and archaeal diversity was assessed by terminal
13 restriction fragment length polymorphism profiling on a selection of samples distributed along
14 chemical gradients. Multivariate statistical analyses explained from 45% to 80% of the spatial
15 variation in microbial diversity, considering only the chemical variables. A selection of trace
16 metals of anthropogenic origin appeared to be strong structural factors for both bacterial and
17 archaeal communities. Bacterial terminal restriction fragment (T-RF) richness correlated
18 strongly with both anthropogenic and natural chemical gradients, whereas archaeal T-RF
19 richness demonstrated fewer links with chemical variables. No significant decrease in
20 diversity was evidenced in relation to chemical contamination, suggesting a high adaptive
21 potential of benthic microbial communities in Toulon Bay.
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51 **Key words:** microbial ecotoxicology, multi-contamination, prokaryotic diversity, marine
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1. INTRODUCTION

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2 Marine coastal areas represent ecotones between continental and marine environments.
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4 Strong and natural coastal gradients (*e.g.* salinity, water inflows, bottom topography,
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6 sedimentation pattern, turbidity and nutrients) are known to shape the diversity of several
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8 organisms, ranging from fishes (Winemiller and Leslie, 1992) to bacteria (Ortega-Retuerta et
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10 al., 2013; Troussellier et al., 2002), including invertebrates (Waterkeyn et al., 2008). As a
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12 result, the functions of coastal ecosystems are largely driven by natural gradients (Sklar and
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14 Browder, 1998). Since coastal areas are of great interest to humans, they also face
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16 increasing human-induced chemical contamination resulting from watershed drainage,
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18 domestic and industrial waste waters and other direct inputs, such as biocides released by
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20 anti-fouling paints or fuel residues from shipping (*e.g.* Colombo et al., 2006; Daskalakis and
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22 Oconnor, 1995; Levin et al., 2009; Oursel et al., 2014, 2013). As a result, marine coastal
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24 areas are characterized by a complex set of chemical factors potentially affecting living
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26 organisms. Consequently, marine coastal ecosystems represent one of the most challenging
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28 environments for understanding the links between chemical composition and biological
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30 diversity.
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35 Benthic archaea and bacteria are key microbial players in ecosystem functioning, because of
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37 their major roles in diverse biogeochemical cycles, particularly through carbon and nitrogen
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39 mineralization (Francis et al., 2005; Jørgensen, 2000) and toxic metals transformation, either
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41 directly (*e.g.* mercury methylation, Parks et al., 2013) or indirectly (*e.g.* formation of sulfide
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43 compounds after sulfide release by sulfate reduction, Jørgensen, 2000). However, little is
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45 known about the links between prokaryotes diversity and the global chemical composition of
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47 the sediment, including both the natural composition and anthropogenic chemical
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49 contaminants, in marine coastal areas. It was evidenced that early diagenesis, which is
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51 controlled by microbial activity, induced modifications to the characteristics of sedimentary
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53 organic matter (*e.g.* Burdige, 2007; Dang et al., 2014a) but also influenced the mobility of
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55 trace elements, such as trace metals/metalloids (*e.g.* Dang et al., 2015a, 2014b; Gendron et
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57 al., 1986), with significant seasonal variations. Moreover, the influence of microbial activity on
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1 metal speciation has been demonstrated, particularly for mercury speciation (Cossa et al.,
2 2014; Duran et al., 2008) and organic tin degradation (Filipkowska et al., 2014; Pougnet et
3 al., 2014) in coastal sediments. Our understanding of the influence of benthic microbial
4 activity on the chemical composition of anthropized coastal sediment is thus progressing
5 well. Nevertheless, the impact of the global chemical composition of sediment on benthic
6 microbial communities is poorly documented. Some bacteria and microalgae were shown to
7 resist or even benefit from high inorganic or organic contaminant concentrations (e.g.
8 Edwards et al., 2013; Gauthier et al., 1992; Louati et al., 2013; Rensing et al., 2000).
9 However, chemical pollution can affect the diversity and functions of prokaryotic communities
10 (Abell et al., 2014; Scott et al., 2014), although the great adaptive skills of prokaryotes after
11 long term exposure to chemical contaminants (Gillan et al., 2014) can lead us to observe no
12 significant change in diversity (Besaury et al., 2014; Gillan et al., 2005), not even higher
13 diversities (Bengtsson et al., 2013) in response to chemical pollution. Such influences result
14 in spatial variability of microbial abundance and/or community composition in the
15 environment, which can alter ecosystem functioning along the contamination gradients (Abell
16 et al., 2014; Scott et al., 2014). Thus, in marine coastal ecosystems, the relative contributions
17 of natural abiotic gradients and anthropogenic and multiple contamination gradients to the
18 spatial variability of microbial diversity need to be further explored in order to better
19 apprehend the ecological consequences of human-induced environmental changes.

20 The present study was held at Toulon Bay, a semi-enclosed coastal ecosystem experiencing
21 very strong and multiple contaminations by metals, metalloids, organometallic and organic
22 pollutants (Cossa et al., 2014; Dang et al., 2015a, 2014b; Pougnet et al., 2014; Tessier et al.,
23 2011). While this ecosystem experiences multiple contamination gradients of several orders
24 of magnitude, its relatively small size greatly limits other abiotic variations such as climate
25 related parameters, hydrology and mean sediment composition (excluding pollutants). An in-
26 depth geochemical characterization of surface sediments and the genetic fingerprinting of
27 archaeal and bacterial benthic communities sampled at numerous stations in Toulon Bay
28 allowed us (i) to address the impact of the global chemical environment and then (ii) to

compare the respective influence of a wide range of both natural and anthropogenic gradients on both local microbial genetic richness and spatial variations in microbial community structure.

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2. MATERIALS AND METHODS

2.1. Study site and sediment sampling

Sediments samples were collected at different locations of Toulon Bay, on the northwest Mediterranean coast. Its semi-close morphology associated with micro-tidal conditions and intermittent freshwater inputs (Nicolau et al., 2012), leads to relatively long water residence times (Dufresne et al., 2014). Consequently, numerous historic (e.g. World War II) and present (1st French navy harbour, nautical traffic, tourism, urbanism, aquaculture, etc.) anthropogenic activities have resulted in a strong multi-contamination of the sedimentary compartment (Cossa et al., 2014; Dang et al., 2015a, 2014b; Pougnet et al., 2014; Tessier et al., 2011) and also of the water column (Jean et al., 2012, Dang et al., 2015b). A map of Toulon Bay including the location of the main towns and anthropogenic activities, is presented in figure 1A. The bay is separated into two basins by a sea wall: Little Bay (LiB) on the northwestern side and Large Bay (LaB) on the southeastern part. Sediments were collected using an interface corer (equipped with 10-cm diameter and 1-m long Plexiglass tube, handled from a navy boat or divers) as previously described (Tessier et al., 2011) to collect sediments at 54 stations covering the whole bay (figure 1).

2.2. Chemical characterization of the sediments

We compiled previously published data on the chemical characterization of the surface sediments, except for the organic pollutants (polycyclic aromatic hydrocarbons (PAHs), polychloro biphenylated (PCBs)). Briefly, the obtained surface sediments (0-5 cm) were analysed for (1) major constituents (major elements, particulate organic and inorganic carbon (POC, PIC), total phosphorus) and minor/trace elements (Dang et al., 2015a, 2014a, 2014b; Tessier et al., 2011); (2) methyl mercury (MMHg) (Cossa et al., 2014); and (3) tin and butyl-tin species (Pougnet et al., 2014). Full description of samples treatment and chemical analysis can be found in the cited references. Briefly, sediments were deep-frozen, freeze-dried, 2-mm sieved and kept deep frozen (-18°C) until analysis. POC and PIC contents were determined using a TOC-V_{CSH} analyzer (Shimadzu) coupled with a SSM-5000A module

(Dang et al., 2014a; Tessier et al., 2011). Major elements and total phosphorus were measured by XRF (Philips PW 2400) equipped with a Rh-tube, on 50- μ m crushed (on carbide tungsten rings mill, NAEF model M02) sediments (Tessier et al., 2011). Minor/trace elements were determined by High Resolution Inductively Coupled Plasma Mass Spectrometer (HR ICP-MS, Element 2, Thermo Finnigan) after aqua regia and microwave (Anton Paar Multiwave 3000) digestion of sediments (Dang et al., 2014b, 2015a; Tessier et al., 2011). Hg contents were determined by an advanced mercury analyzer AMA 254 (Leco Corporation) (Tessier et al., 2011). MeHg was analysed after propylation and isotopic dilution and detection by gas chromatography-ICP-MS (GC/ICP-MS, GC-Focus with X-series, Thermo Electron) (Cossa et al., 2014). Butyl-tin species contents were determined by GC/ICP/MS (Thermo X7 Series II) after extraction of butyl-Sn species by acetic acid (Pougnnet et al., 2014). Moreover, 25 surface sediment samples from LiB (figure 1) were analysed for 17 PAHs (Acenaphthene, Anthracene, Benz[a]anthracene, Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[ghi]perylene, Benzo[k]fluoranthene, Chrysene, Dibenz[a,h]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-cd]pyrene, Naphthalene, Phenanthrene, Pyrene, 2-methylfluoranthene, 2-methylnaphthalene) and 8 PCBs (CB-28, CB-31, CB-52, CB-101, CB-118, CB-138, CB-153, CB-180), accordingly to the French standard AFNOR XP X33-012. Briefly, PAHs were extracted from 4 g of sediment using a mixture of hexane/acetone (50:50) assisted by ASE (ASE 200, Dionex), the obtained extract was then purified by solid-phase extraction (SPE, ASPEC XL, Gilson), concentrated (50°C under N₂ flux), adjusted to 5 mL with acetonitrile/water (50/50) and PAHs concentrations were analysed by HPLC (Shimadzu, Prominence UFLC) with UV and fluorescence detection. Concerning PCBs, they were extracted from 7 g of sediments by using a mixture of hexane/acetone (50:50) assisted by ASE (ASE 200, Dionex) in the presence of 1g of Cu powder. The obtained extract was then purified on SPE (ASPEC XL, Gilson), concentrated (50°C under N₂ flux), and adjusted to 1 mL with hexane. CBs concentrations were analysed by GC/MS (Thermoelectron, Polaris Q) in negative chemical ionization mode. The accuracy

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2 of these protocols was validated using certified material (MRC 1941b and MRC 1944 for
3 PAHs and CBs, respectively).
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6 **2.3. DNA extraction and prokaryotic fingerprinting**

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8 DNA was extracted from aliquots of lyophilized sediments (kept frozen at -20°C) from 38
9 samples from among the 54 described above (figure 1), covering the whole range of main
10 sediment characteristics and chemical contaminants levels. Extractions were performed
11 using the Power Soil DNA Extraction Kit (Mobio Laboratories, Carlsbad, CA) following the
12 manufacturer's recommendations. Terminal Restriction Fragment Length Polymorphism (T-
13 RFLP) profiling was conducted on each sample after 16S rRNA gene amplification using
14 primers 8F (Lane, 1991)/519R (Frank et al., 2013) labeled with FAM (5-carboxyfluorescein)
15 and HEX (6-carboxy-1,4-dichloro-2',4', 5', 7'-tetrachlorofluorescein) fluorochromes
16 respectively for Bacteria and 344F (Raskin et al., 1994)/915R (Stahl and Amann, 1991)
17 labelled with FAM and HEX fluorochromes respectively for Archaea using AmpliTaq Gold®
18 DNA Polymerase (Life technologies). For PCR amplification an initial denaturation step at
19 94 °C for 5 min was followed by 35 cycles of 94 °C denaturation for 45 s, 56 °C annealing for
20 45 s, and 72 °C extension for 1 min, and concluded with an extension at 72 °C for 10 min.
21 PCR amplicons were cleaned using the GFX PCR DNA Purification kit (GE Fisher) and
22 digested with the restriction endonucleases HinfI for Bacteria and TaqI for Archaea (37°C, 3
23 h). T-RFLP profiles were obtained with a 3130xl Genetic Analyzer (Applied Biosystems)
24 following the manufacturer's recommendations and analysed with GeneMapper® Software
25 (Applied Biosystems). Using T-Rex software (Culman et al., 2009) raw data were normalized
26 to account for differences in the amount of DNA loaded and terminal restriction fragments (T-
27 RFs) assigned to produce the final matrix.
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53 **2.4. Data analysis and statistics**

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55 The high spatial resolution maps allowed the establishment of surface-weighted average
56 (SWA) trace metal/metalloids contents for Toulon Bay, *i.e.* the most representative
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1 concentration values on the entire bay scale (Dang et al., 2015a; Larrose et al., 2010;
2 Tessier et al., 2011). The kriging gridding method of Surfer software (9.9 version) with default
3 parameters was used to generate contour map and Matlab software (7.8 version) was used
4 for data processing.
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8 Using a principal component analysis (PCA) on 2 chemical characteristics (PIC, POC) and
9 31 major/minor/trace elements (Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Li,
10 Mn, Mo, Ni, P, Pb, Rb, S, Sb, Sn, Sr, Ti, Tl, U, V and Zn) from 130 surface and deep
11 sediments from Toulon Bay, Dang et al. (2015a) have evidenced 6 groups of elements: (1)
12 the "marine" originated elements (Ca, PIC and Sr), (2) the "terrigenous" elements, mainly
13 representing clays (Al, Be, Cs, Fe, K, Li and Rb), (3) "inorganic contaminants", i.e. elements
14 mainly of anthropogenic origins (Ag, Ba, Bi, Cd, Cu, Hg, P, Pb, Sb, Sn, Ti and Zn), (4)
15 inorganic trace elements having mainly "natural" origin, from terrigenous inputs (Co, Mo, Ni,
16 Tl, U and V), (5) elements showing "mixed" anthropogenic and terrigenous origins (As, Cr,
17 Mn and S), and (6) the POC. To illustrate these co-variations, the observed relationship
18 between PIC and Ca, Rb vs. Al, Bi vs. Pb, Co vs. Ni and As vs. Cr in the surface sediments
19 dataset considered in the present study are shown in Fig. SI-1. In order to reduce the
20 number of chemical variables for further statistical analysis, the elements of "marine" origin
21 were then only represented by Ca, the "terrigenous" elements by Al and Fe, and the trace
22 elements of "natural" origin by Co and Ni, when POC, "inorganic contaminants" and trace
23 elements of "mixed" origin were all taken into consideration. Then, a 7th group of
24 "organometallic contaminants" was defined using: monomethylmercury (MMHg), tributyltin
25 (TBT) and the sum of butyltin-species (Σ BT). Significant relationships observed between TBT
26 and Σ BT, and MMHg and Hg (Fig. SI-2) were evidenced by Pougnet et al. (2014) and Cossa
27 et al. (2014) and suggested the strong influence of microbial communities on the
28 sedimentary dynamics of these species. Finally, an 8th group included the "organic
29 contaminants" (PAHs and PCBs). Considering the significant correlations (R^2 ranging from
30 0.86 to 0.98 and from 0.95 to 0.99 for PAHs and PCBs respectively, data not shown)
31 observed between each of the 17 measured PAHs and their sum (Σ PAH), and between each
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1 of the 8 measured PCBs and their sum (Σ PCB), the present study only considered the
2 following chemical variables: Σ PAH, Σ PCB, Fluoranthene and CB-153 (being the most
3 abundant PAH and PCB, accounting on average for 16% and 29% (N=26) of the Σ PAH and
4 Σ PCB, respectively; figure SI-3).

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9 This clustering of chemical variables highlighted five groups corresponding to the sediment
10 type ("marine", "terrigenous", POC₇, and inorganic trace elements of "natural" or "mixed"
11 origin) and three groups of anthropogenic contaminants ("inorganic", "organometallic" and
12 "organic" elements/compounds), resulting in 29 representative chemical variables. The
13 content ranges, median content and SWA (only for the 23 variables measured on all the
14 sediments samples) values of these chemical variables are summarized in table 1.

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Enrichment factors (EF) (Hilton et al., 1985) were calculated for trace metals/metalloids
contaminants in order to distinguish anthropogenic pollution from the natural content of the
sediment as described in Tessier et al. (2011). Briefly, the measured trace elements contents
were normalized to the Al contents of the samples, and then compared with the geochemical
background of the studied area for the same normalized metal content, according to the
following equation:

$$EF = \frac{(ME/Al)_{sample}}{(ME/Al)_{background}}$$

where Me is the content of the corresponding trace metal/metalloid. The geochemical
background was here estimated as the average of the three lowest calculated Me/Al ratios
from 146 samples originating from surface and deep sediments (including ²¹⁰Pb-dated core
sediments corresponding to the lowest contamination records, from Dang et al., 2015a and
Tessier et al., 2011) of Toulon Bay. According to Sutherland (2000), significant enrichment
levels are reached if 5 < EF < 20, and very high enrichment levels if EF > 20.

The toxic potential of the sediment was evaluated through comparison of the contents to
effects range-low (ERL) and effects range-medium (ERM) guidelines, initially determined
through toxicity evaluation on benthic invertebrates exposed to single contaminations (Long
et al., 1995). Then, in order to assess the global toxic potential of the multi-contaminated

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sediments of Toulon Bay, the mean ERM quotient (m-ERM-Q) was calculated as described in Long et al. (1998), according to the following equation:

$$m - ERM - Q = \frac{\sum_{i=1}^n (C_i / ERM_i)}{n}$$

where C_i is the sediment content of compound i , ERM_i is the ERM for compound i and n is the number of compounds.

Local (alpha) diversity indexes (number of terminal restriction fragments (T-RF richness), Pielou's Evenness and Shannon H') were computed using PRIMER 6 software (v6.1.13, Clarke, 1993).

Spatial variations of both chemical composition and microbial communities' T-RF structure (beta-diversity) were analysed through quantitative distance analyses, conducted using PRIMER 6 software. Chemical Gower similarities were computed from median normalized substance contents in order to properly compare minor and major element content² variation. Biological Bray-Curtis similarities were computed from $\text{asin}(\sqrt{x})$ normalized T-RFLP data (Legendre and Legendre, 1998). Differences between samples originating from LiB or LaB were visualized through principal coordinate analysis (PCoA) and tested by one-way permutational multivariate analysis of variance (PERMANOVA) with Rstudio (RStudio Team, 2015, package *vegan*).

Correlations between biological and chemical similarities were assessed by Mantel's test using XLStat (version 2010.3.03, Addinsoft, Paris, France). Other correlations were tested with Spearman's correlation test using Rstudio (RStudio Team, 2015, packages *stats* and *psych*)

Variation partitioning through direct multivariate canonical correspondence analysis (CCA) was used to compare the respective contributions of anthropogenic contaminants and sediment type to the spatial variability of archaeal and bacterial community structure. In order to avoid using multicollinear variables and thus overestimating contributions to the biological variance, covariance analyses were performed and variables presenting variance inflation

factors higher than 10 were excluded from the dataset. Covariance analysis and CCA were performed with Rstudio (RStudio Team, 2015, packages *vegan*, *HH* and *usdm*).

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3. RESULTS

3.1. *Abiotic characterization of the sediments*

A set of chemical gradients was observed in surface sediments of Toulon Bay. Groups related to sediment origin and natural and mixed trace elements presented narrow to moderate content gradients (table 1), with Al (figure 2A) and Ca (figure SI-4A) presenting clear terrestrial-marine gradients resulting in an opposite relationship between these 2 elements (figure SI-5). Among inorganic contaminants (ex. Cd, figure 2B), the strongest spatial variations in concentrations was observed for Cu, Hg, Sn and Zn, with about 55- to 900-fold content ranges (table 1). Organo-metals also presented very wide content gradients, with a 13500-fold range for TBT and an approximately 80-fold range for MMHg (table 1). Organic contaminations followed moderate to strong gradients with approximately 15 to 89-fold content ranges for Σ PAH (figure SI-4B), fluoranthene, POC, CB-153 (figure 2C) and Σ PCB, respectively (table 1). Most of the natural or mixed trace elements and inorganic, organometallic or organic contaminants presented similar distribution, their content values being the highest in the northern part of LiB and gradually decreasing toward the south-east and the open sea (for representative examples, see figures 2B, 2C and SI-4A). Tin, POC fluoranthene and Σ PAH did not follow this trend, presenting more random variations (figure SI-4B).

Considering most of these representative physical-chemical parameters together (except for TBT, Σ BT, fluoranthene, Σ PAH, CB-153 and Σ PCB, which were not measured in all of the sampling sites), spatial variations in the global chemical signature of the sediment (*i.e.* considering all 8 of the clusters identified above) were assessed by calculating Gower similarities. This distance analysis demonstrated important variations of sediment chemical composition with reference to the less contaminated sites (two stations located south-east and south-west of Toulon bay, respectively, away from most of the human activities centers), with an offshore-to-coast gradient following the distribution of the main contaminants (figure 2D). Very important shifts of chemical signature were especially recorded in LiB, the most

1 anthropized area, resulting in a very low degree of similarity (decreasing to 28%) compared
2 with the mouth of the bay. Similar gradients were globally observed when computing Gower
3 distances for the chemical signatures of the sediment type and the anthropogenic
4 contaminants, with significant local shifts observed mainly in the northern LiB and near the
5 coasts of St. Mandrier's peninsula (figure SI-4C and D). Specific distance assessments with
6 fewer sampling points to include organic contaminants (24 sampling sites) or TBT and Σ BT
7 (29 sampling sites) confirmed this trend (data not shown).
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10 **3.2. Contamination levels and potential toxicity**

11 In order to better evaluate sediment contamination by trace metals, Al-normalized
12 enrichment factors (EF) were calculated to compare the surface sediment concentrations of
13 Ag, As, Ba, Bi, Cd, Co, Cr, Cu, Hg, Ni, P, Pb, Sb, Sn, Ti and Zn with the geochemical
14 background of Toulon bay (table 1). Mercury was clearly the most enriched inorganic
15 contaminant in these sediments, reaching an EF as high as 1200 and presenting very high
16 enrichment levels (EF > 20) in approximately 80% of the surface sediment area of Toulon
17 Bay (table 1). A few other inorganic contaminants (Ag, Bi, Cu, Pb, Sb, Sn, and Zn) presented
18 significant enrichment levels (5 < EF < 20) over 10% to 90% of the surface of Toulon Bay
19 and very high local enrichment levels, reaching up to 50 and 54 for Sn and Cu, respectively.

20 As detailed in table 1, the highest recorded concentrations of five inorganic contaminants
21 (As, Cu, Hg, Pb and Zn) and of two organic contaminants (fluoranthene and Σ PCB)
22 surpassed their ERM values. Moreover, approximately 50% of the surface of Toulon Bay
23 presented Hg contents overpassing the corresponding ERM. Then, m-ERM-Q was
24 calculated in order to compare the toxic potential of the different sediment samples. When
25 considering only trace metals, 26.6% of the whole surface of Toulon bay presented m-ERM-q
26 values above 0.5, representing a probability of being toxic of approximately 50% or higher.
27 Moreover, more than 80% of the surface presented m-ERM-Q values above 0.5. According
28 to spatial variation in metals-based m-ERM-Q, the highest toxic potentials were observed in
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the northern part of LiB (figure 2E). Including organic pollutants also confirmed this trend (figure SI-4E).

3.3. Local microbial diversity variations

Bacterial T-RF richness ranged between 14 and 40 OTUs. It appeared to be higher in LiB, and decreased gradually through LaB where it reached its minimum before increasing slightly towards the open sea (figure 3A). Archaeal T-RF richness appeared to be smaller with 4 to 13 T-RFs, and varied in a more chaotic way with important changes between very close sampling stations (figure 3B). Shannon indexes calculated from T-RFLP data provided very similar results (H' ranging from 1.34 to 2.43 for archaea and from 2.60 to 3.52 for bacteria) and were very strongly correlated to richness ($R^2 = 0.96$ for archaea and 0.94 for bacteria). Evenness demonstrated very low spatial variations (ranging from 0.91 to 0.995 for both bacteria and archaea) and was not correlated with Shannon H' variations ($R^2 < 0.1$ for both archaea and bacteria), thus highlighting the importance of richness in bacterial and archaeal Shannon index variations among sediment samples. Hence, local microbial diversity will be represented by genetic richness in further analyses.

Bacterial T-RF richness was positively correlated with parameters related to “terrigenous” elements (Al, Fe), one “natural” trace element (Ni), trace elements of “mixed” origin (As, Cr and S), some anthropogenically enriched toxic trace metals (Ag, Cu, Hg and Zn), organometallic contaminants (MMHg, TBT and Σ BT), some organic contaminants (CB-153, Σ PCB) and metal-based m-ERM-Q, whereas it was correlated negatively with marine elements (Ca), Co and Ni ($p < 0.05$, table 2).

Archaeal genetic richness proved to be linked to fewer environmental parameters: it was also positively correlated with terrigenous elements (Al, Fe), one trace element of mixed origin (Cr), some anthropogenically enriched toxic trace metals (Bi, Cu, Hg, Pb and Zn), organometallic contaminants (MMHg, TBT and Σ BT) and m-ERM-Q values ($p < 0.05$, table 2).

1 Hence, in Toulon Bay, both bacterial and archaeal T-RF richness appeared to be higher in
2 sediments influenced by terrestrial inputs, enriched in many toxic substances, and thus
3 presenting a high toxic potential. However, for most of the chemical substances presenting
4 significant correlations with the T-RF richness of both microbial communities, the correlations
5 were weaker and less significant for archaea. Particulate organic carbon, Mn and P contents
6 in the sediments did not appear to be linked with either bacterial or archaeal T-RF richness
7 ($p > 0.05$, table 2)
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10 **3.4. Spatial variations in microbial community structure**

11 Beta- diversity analysis (PERMANOVA) of the bacterial community highlighted a clear
12 segregation ($p = 0.001$) of the samples originating, on one hand, from LiB and, on the other
13 hand, from LaB and towards the open sea (figure 4A). With reference to the less
14 contaminated sites located outside of Toulon Bay, the bacterial similarity appeared very low
15 (decreasing to approximately 20%) upon entry into LaB, then increased slightly but varied
16 strongly again between very close stations westward (figure 3C). Complementary analysis
17 with reference to the most contaminated site (MIS station, in the northern part of LiB, figure
18 SI-4F) demonstrated very strong differentiation towards the south of the bay, with low
19 similarity (decreasing to 50%) between sites distant from barely more than 1km. In contrast,
20 no evident clustering according to geographical origin ($p = 0.052$) could be observed for the
21 archaeal community (figure 4B). The archaeal similarities were globally higher and less
22 variable, with the strongest shifts being observed in LiB and near the mouth eastward (figure
23 3D).
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26 Both archaeal and bacterial similarities were negatively correlated with the geographical
27 distance between sites ($n = 703$, archaea: $r = -0.286$, $p < 10^{-4}$, bacteria: $r = -0.285$, $p < 10^{-4}$),
28 and positively correlated to the sediment type's chemical signature ($n = 703$, archaea: $r =$
29 0.195 , $p < 10^{-4}$, bacteria: $r = 0.209$, $p < 10^{-4}$). However, the spatial variation in the global
30 chemical signature only appeared very weakly correlated with the bacterial similarities ($n =$
31 703 , $r = 0.094$, $p = 0.014$) and not significantly correlated with the archaeal similarities ($n =$
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703, $p > 0.05$). The anthropogenic contaminants' chemical signature did not demonstrate any significant correlation with biological similarities ($n = 703$, $p > 0.05$). The same trends were recorded with reduced datasets including BT species ($n = 276$) or organic contaminants ($n = 171$), while archaeal similarities only correlated significantly with the geographic distances between sites (data not shown).

When considering all of the sampling stations ($n = 38$), the combination of sediment type (represented by Ca, Co, As, Cr, Mn, S and POC) and anthropogenic contaminants (Ba, Cd, Hg, P, Sb, Sn and Ti) explained 46% of the bacterial community's structural variation, each of them accounting for 26% on its own. Similarly, this dataset explained 51% of the archaeal community's structural variation, with anthropogenic contaminants explaining a little less than sediment type (26% and 26%, respectively). Including BT species did not significantly increase the contribution of the anthropogenic contaminants, since TBT and Σ BT never explained more than 5% of the variability of the archaeal and bacterial communities' structure, far less than the other contaminants, which contributed up to 9% in the same dataset ($n = 24$). However, the global statistical contribution of the chemical dataset to the biological variation rose to 60% for both bacteria and archaea. Similarly, CB-153 and Σ PCB proved to be the least important statistical contributors to biological variation, with contributions ranging between 4% and 6%, whereas other single contaminants could explain up to 13%. On the contrary, fluoranthene and Σ PAH contributed significantly (between 6% and 10%), and appeared to be among the four best statistical contributors of all the contaminants in the corresponding dataset ($n = 19$). Within this reduced dataset, the global statistical contribution to biological variation reached 85% and 78% for bacteria and archaea, respectively.

4. DISCUSSION

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2 Marine coastal areas are subject to various natural and anthropogenic gradients. The benthic
3 compartment of Toulon Bay illustrates well this general observation with gradients of POC
4 content, sediment origin and multiple contaminations. In the literature, previous attempts to
5 understand the contributions of the various factors shaping prokaryotic communities in
6 different ecosystems mainly focused on the relative contributions of bottom-up and top-down
7 variables to better understand food-web functioning (e.g. Berdjeb et al., 2011; Salcher, 2013;
8 Van Wambeke et al., 2009) or on the influence of chemical pollutants through
9 ecotoxicological approaches (e.g. Besaury et al., 2014; Duran et al., 2015; Kostka et al.,
10 2011; Paisse et al., 2011; Quillet et al., 2012; Rasmussen and Sorensen, 2001). In this
11 study, we chose to investigate the influence of the abiotic environment as a whole on
12 microbial communities by considering together both potentially toxic contaminants and
13 natural substances from various origins. The usefulness of such a global approach was
14 demonstrated in this study by the major contribution of the chemical composition of the
15 sediment in shaping benthic microbial communities of Toulon Bay. Indeed, from
16 approximately 45% to more than 80% of the variability of bacterial and archaeal community
17 structure could be statistically explained by a selection of chemical variables. Thus, a
18 significant part of this variability remained statistically unexplained in our study,
19 corresponding to some abrupt shifts that were observed in close and chemically similar
20 sampling sites. This could be partly due to biotic factors, such as predation or viral lysis
21 pressures, which are well known for their influence on prokaryotic community abundance and
22 structure (Ben Said et al., 2015; Louati et al., 2013; Ram et al., 2014; Sauret et al., 2015) but
23 were not investigated in this work.

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51 Investigating global chemical signatures also proved to be valuable through the significant
52 links that were observed between biological and chemical distances. Interestingly, neither the
53 global chemical distance between the different sampling sites, nor the distance related to
54 anthropogenic contaminants appeared strongly linked to biological distances, whereas
55 geographical distances demonstrated stronger links with biological ones. This observation
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1 tends to demonstrate that, in this contaminated sediment, local selective pressures are
2 stronger than the dispersal rate of benthic microbial communities, in spite of the very limited
3 geographic scale. The positive relationship between biological distances and the chemical
4 distance related to sediment type (including its marine or terrestrial origin) suggests that
5 benthic microbial dispersal would be limited to sediment transport processes, which could
6 promote strong spatial differentiation. However, the high connectivity through the water
7 column within this marine coastal area, with a mean annual water exchange time of 3.4 days,
8 makes this hypothesis unlikely (Dufresne et al., 2014). Thereafter, high dispersal but low
9 survival of benthic microorganisms, because of major chemical shifts between the origin and
10 end point environments, appears to be a more reliable hypothesis. Future deep taxonomic
11 investigations will help to test these two hypotheses.

12 Thus, when facing the complexity of chemical variable interactions, such as the antagonistic
13 effects of nutrients and pollutants, an in-depth geochemical characterization of the
14 environment appears useful for providing a holistic and detailed chemical signature of the
15 samples. Then comparing this global chemical signature to other environmental constraints,
16 such as biotic interactions, appears to be a promising approach to understanding the ecology
17 of microorganisms and the influence of the human footprint on ecosystem functioning.

18 Another striking result of this study came from multivariate analyses and linear regression
19 analyses to evaluate the relative contributions of different variable classes to the observed
20 biological variation. Among the abiotic parameters measured, metallic contamination clearly
21 appeared to be an influential factor in shaping benthic prokaryote community structure in
22 Toulon Bay. Indeed, as demonstrated by partial CCA results, at least 26% of the spatial
23 variation in bacterial and archaeal community structure could be explained by a selection of
24 trace metal contaminants. The gradient of benthic contamination in Toulon Bay by such
25 pollutants includes both very low concentrations (*i.e.*, close to the geochemical background
26 of the region) and dramatic enrichments (*e.g.*, Hg contamination), when compared with other
27 ecosystems (for a more detailed comparison, see Tessier et al., 2011). Then, considering the

1 panel of potential toxic effects these pollutants are known to have on microorganisms (for
2 review, see Gadd, 2000; Nies, 1999), observing biological gradients in Toulon Bay appears
3 logical. These results supported the intention to use bacterial communities as sensitive
4 indicators of contaminant stress for use as an environmental monitoring tool (Sun et al.,
5 2012). However, we did not observe any reduction in prokaryotic T-RF richness where trace
6 metals and organometallic substances were the most enriched. On the contrary, we
7 observed higher bacterial richness in the north-western bay, the most polluted area. This
8 observation was confirmed by numerous positive, strong and significant correlations of T-RF
9 richness with the concentrations of metallic and organometallic pollutants. In the same way,
10 strong and positive correlations were also observed between T-RF richness and the toxic
11 potential of the sediment estimated by the m-ERM-Q. However, caution is needed as this
12 index is based on the sensitivity of macroorganisms rather than that of microorganisms (Long
13 et al., 1998). Therefore, m-ERM-Q is not well suited for predicting microbial community
14 responses to chemical contaminations. Whereas metals and organometallic substances are
15 well known for their toxic effects, their high concentrations were associated with a higher
16 microbial richness. This striking relation tends to contradict the global erosion of marine
17 biodiversity caused by anthropogenic inputs in the marine environment (Johnston and
18 Roberts, 2009). However, the responses of microbial diversity to contaminants are more
19 complex than those of marine macrobiota and similar observations have been reported in
20 other marine coastal sediments (Duran et al., 2015; Li et al., 2013; Paissé et al., 2008).

21 One way to explain this contradiction relies on the intermediate disturbance hypothesis
22 (IDH), which predicts the highest diversity for disturbances of intermediate frequency and
23 intensity (Connell, 1978). Indeed, although concentrations of the pollutants observed in
24 Toulon Bay can strongly disturb macroorganisms due to bioaccumulation and lower
25 reproduction rates, they may induce a lower disturbance to the microbiota that grow faster
26 and is able to resist and transform metal and organo-metallic substances (Nies, 1999).
27 Moreover, the most enriched pollutants in the sediments of Toulon Bay are historical
28 contaminants that have been present in excess for several decades (Pougnnet et al., 2014;

1 Tessier et al., 2011). This stability of the contamination level at the scale of microbial lifetime
2 could therefore no longer represent a disturbance with respect to the IDH. Indeed, the
3 bacterial community might have evolved and could now be particularly adapted to such an
4 environmental stress, as observed in similar ecosystems impacted by metallic pollutions (*e.g.*
5 Besaury et al., 2014). In chronically polluted systems, diversity indexes may be a non-
6 adapted indicator of ecosystem stress as it can recover due to the divergence and
7 proliferation of tolerant species (Gillan et al., 2005).
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17 Organic contaminants appeared to be a less influential environmental stress on benthic
18 prokaryote communities than metallic and organo-metallic contaminations in Toulon Bay
19 sediments by statistic disentangling. Indeed, only a few links between organic contamination
20 and prokaryote T-RF richness were observed in the sediment of Toulon Bay. Moreover, the
21 contribution of organic contaminants to beta diversity patterns appeared limited. This
22 observation is quite surprising when considering the relatively high concentrations measured
23 in comparison to other sites and with reference to sediment quality guidelines (*e.g.* Gao et
24 al., 2013; Long et al., 1998; Wolska et al., 2014). Moreover, several studies reported
25 decreases, or at least very significant shifts, in microbial diversity in response to PAHs or
26 PCBs contamination (*e.g.* Correa et al., 2010; Guazzaroni et al., 2013). This could be
27 explained by the difference of more than one order of magnitude observed between the
28 respective extents of contamination gradients of metallic and organic pollutants. The
29 influence of organic contamination could thus be hidden by that of toxic trace metals.
30 Nevertheless, as for trace metals, organic contaminations appeared to be positively related
31 to prokaryote T-RF richness in Toulon Bay. Similar observation were previously reported in
32 other ecosystems (Bengtsson et al., 2013), which could indicate a characteristic feature of
33 contaminated sites with multiple contaminant types. Indeed, microorganisms are also known
34 for their ability to grow on organic substrates, and especially PAH. Organic and more or less
35 biodegradable contamination could thus increase the diversity of carbon resources for
36 heterotrophic prokaryotes, creating new ecological niches. Thus, in agreement with IDH,
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organic contamination could partly explain the higher microbial richness found in the north-western part of the bay as previously demonstrated (Bengtsson et al., 2013). Organic contamination could actually counterbalance the degradation of microbial diversity that could occur because of toxic metal enrichment, and finally reduce the extent of disturbance of these microorganisms.

The chemical gradients observed in the sediment of Toulon Bay did not present the same relations with the archaeal and bacterial benthic communities. This observation suggests differential responses of bacteria and archaea to this kind of chemical contamination. The spatial variations of archaeal T-RF richness were weaker than for bacteria, without a significant increase in the most polluted area, and demonstrated fewer and weaker links to the abiotic variables recorded, especially those corresponding to the sediment origin or to biologically relevant substances (POC, S and Mn). Although the low number of archaeal T-RFs detected could raise some methodological concerns, such as partial recovery of the archaeal community, the validation of the method in various previous studies (*e.g.* Stevenson et al., 2014) tends to invalidate this argument. Moreover, the weak relation between archaeal T-RF richness and the chemical substances measured is counterbalanced by the explanation of 26% to nearly 50% of the spatial variation of archaeal community structure by anthropogenic contaminants, as observed for the bacterial community. This observation could indicate that, whether or not the richness of the community was only poorly affected, its composition was likely more affected. From an ecological point of view, metal resistance in archaea and bacteria involves similar enzymatic responses (Bini, 2010), but archaea are known to predominate in extreme environments, especially where they are exposed to particularly high metal stress as in extremely acidic ecosystems (Baker and Banfield, 2003; Bini, 2010; Edwards et al., 2000). The question arises as to whether archaeal communities could present different and more widespread adaptive strategies than bacterial communities when facing multi-contaminations, especially the combination of organic, organometallic and inorganic contaminants. Further inventories of structural and functional diversity of bacteria

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2 and, above all, archaea are now required to explain the different trends we observed in this
3 study between these communities.
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6 Whether inorganic, organometallic or organic, the influence of a pollutant or chemical cocktail
7 depends on its bioavailability. Although not studied in this work, Dang et al. (2014b, 2015a)
8 have previously demonstrated that trace metals and metalloids could be highly mobilized
9 from particles to pore water in surface sediments (0-10 cm), and that this mobilization could
10 vary throughout the year. This suggests a potentially high bioavailability of inorganic
11 contaminants in the sediment of Toulon Bay, and tends to support the hypothesis of well-
12 adapted communities. Thus the observed link between the mobility of inorganic contaminants
13 and diagenesis activity throughout the seasons (Dang et al., 2014b, 2015a) also suggests
14 that, in addition to being well adapted, microbial communities could drive the fate of these
15 contaminants in the sediments of Toulon Bay. Now, investigating microbial community
16 composition and focusing on functional communities with higher resolution methods under
17 controlled conditions could help to shed the light on these different interactions between
18 chemical contamination and microbial communities, as well as on potential cross-interactions
19 between contaminant types.
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42 **5. CONCLUSION**

43 Through this study, we have demonstrated the strong influence of the sediments' chemical
44 signature on marine coastal benthic prokaryotic community diversity. In the sediments of
45 Toulon Bay, the influence of such a chemical signature appeared to be mainly related to
46 contamination gradients of trace metal and organo-metallic compounds, which proved to be
47 strongly related to spatial variation in alpha and beta diversity of bacteria, and to a lesser
48 extent, of archaea. Although bacterial and archaeal diversity appeared to be linked to this
49 chemical signature, the major contamination gradients in Toulon Bay surface sediments did
50 not appear to have resulted in diversity loss. On the contrary, bacterial richness seemed to
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be higher in the most polluted areas. The underlying ecological adaptive processes and the cross interactions between the multiple pollutant classes now require further clarification.

The current socio-economical context leads more and more scientists to focus on the consequences of human-related inputs for the environment, and especially on the threat they represent to living organisms and natural communities. For instance, it was recently demonstrated that the high Pb levels observed both in farmed and wild mussels from Toulon Bay, mostly originate from Pb sediment contamination, evidencing a clear link between the contaminated abiotic compartment and biotic species (Dang et al., 2015b). However, microorganisms are also well known for their role in the transformation of chemical contaminants, and especially in the geochemistry of toxic trace metals. Our work, in which no apparent toxic effect was observed in spite of very high contamination levels, reminds us how difficult it can be to evaluate which component, *i.e.*, the chemical or the microbiological environment, influences the other to the greatest extent, even in multi-contaminated sites. The microbial contribution to the geochemical cycles of trace metals is still of major interest, and this study illustrates the need for better understanding to fully elucidate the consequences of toxic trace metal inputs to biodiversity. Further in-depth analyses of structural and functional diversities within microbial communities will help in identifying key species and adaptive mechanisms.

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Table1

[Click here to download Table: table 1.docx](#)**Table 1.** Chemical characterization of the Toulon bay surface sediments.

Variable groups (unit)	Chemical parameters	N	Content range (median)	SWA content	EF range (median)	Surface (%) per EF level				ERL ($\mu\text{g g}^{-1}$)	ERM	Surface (%) per ER level		
						EF < 1.5	1.5 < EF < 5	5 < EF < 20	EF > 20			Content < ERL	ERL < content < ERM	Content > ERM
Marine ($\mu\text{g.g}^{-1}$)	Ca	54	84000 - 320000 (140000)	191000	-	-	-	-	-	-	-	-	-	-
Terrigenous ($\mu\text{g.g}^{-1}$)	Al	54	4700 - 57000 (29000)	22000	-	-	-	-	-	-	-	-	-	-
	Fe	54	5400 - 35000 (19000)	16000	-	-	-	-	-	-	-	-	-	-
Natural ($\mu\text{g.g}^{-1}$)	Co	54	1.9 - 10 (6.2)	5.56	1 - 6.5 (1.6)	15.8	83.7	0.5	0.0	-	-	-	-	-
	Ni	54	5.1 - 40 (22)	17.7	1 - 1.9 (1.3)	91.4	8.6	0.0	0.0	20.9	51.6	65.0	35.0	0.0
Mixed ($\mu\text{g.g}^{-1}$)	As	54	6.2 - 80 (21)	19.8	0.7 - 21 (2.1)	2.2	92.1	5.7	0.0	8.2	70	1.3	98.5	0.1
	Cr	54	12 - 95 (38)	31.9	1.5 - 3.9 (2)	0.9	99.1	0.0	0.0	81	370	99.5	0.5	0.0
	Mn	54	99 - 930 (150)	185	-	-	-	-	-	-	-	-	-	-
	S	54	2900 - 17000 (6100)	5470	-	-	-	-	-	-	-	-	-	-
Inorganic contaminants ($\mu\text{g.g}^{-1}$)	Ag	54	0.061 - 2.8 (0.32)	0.31	0.9 - 22 (3.4)	1.6	87.4	11.0	0.1	1.0	3.7	94.4	5.7	0.0
	Ba	54	30 - 480 (150)	134	1.7 - 5.2 (2.6)	0.0	99.7	0.3	0.0	-	-	-	-	-
	Bi	54	0.17 - 4.6 (0.82)	0.63	1.5 - 25 (5.5)	0.0	48.4	51.5	0.1	-	-	-	-	-
	Cd	54	0.07 - 1.7 (0.21)	0.202	1.2 - 14 (2.3)	2.3	88.2	9.6	0.0	1.2	9.6	99.6	0.4	0.0
	Cu	54	5.6 - 730 (65)	60.5	1 - 54 (6.4)	1.3	50.3	46.6	1.7	34	270	43.3	54.8	1.9
	Hg	54	0.03 - 27 (1.6)	1.54	1.7 - 1200 (77)	0.0	0.7	20.3	79.1	0.15	0.71	11.6	40.7	47.8
	P	54	290 - 930 (540)	474	2.1 - 16 (2.9)	0.0	80.8	19.2	0.0	-	-	-	-	-
	Pb	54	15 - 470 (99)	77	1.6 - 38 (9.6)	0.0	9.8	89.6	0.6	46.7	218	33.9	64.6	1.5
	Sb	54	0.18 - 5.5 (0.62)	0.786	1.8 - 44 (4)	0.0	47.9	50.1	2.1	-	-	-	-	-
	Sn	54	0.94 - 120 (10)	12.9	1.3 - 50 (5.2)	2.6	37.7	55.8	4.0	-	-	-	-	-
	Ti	54	91 - 640 (370)	320	1.1 - 3.4 (1.7)	21.4	78.6	0.0	0.0	-	-	-	-	-
Zn	54	27 - 1500 (150)	136	1.7 - 31 (4.5)	0.0	77.1	22.6	0.4	150	410	68.6	28.4	3.1	
Organometallic contaminants (ng.g^{-1})	MMHg	54	0.18 - 15 (2.5)	1.99	-	-	-	-	-	-	-	-	-	-
	TBT	29	0.2 - 2700 (24)	-	-	-	-	-	-	-	-	-	-	-
	Σ BT	29	0.86 - 4500 (140)	-	-	-	-	-	-	-	-	-	-	-
Organic contaminants ($\mu\text{g.g}^{-1}$)	CB-153	25	0.013 - 0.42 (0.05)	-	-	-	-	-	-	-	-	-	-	-
	Σ CB	25	0.018 - 1.6 (0.11)	-	-	-	-	-	-	0.023	0.18	-	-	-
	fluoranthene	25	0.34 - 6.2 (2.1)	-	-	-	-	-	-	0.60	5.1	-	-	-

Table 2. Spearman's correlation tests between archaeal or bacterial T-RF richness and chemical variables. Numbers represent ρ values of statistically significant correlations. n=38 for most of the correlation analyses, except for BTs and organic compounds for which n = 24 and 19, respectively. ns: non significant. *: $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Variable groups	Chemical parameters	Archaea	Bacteria
Marine	<i>Ca</i>	ns	-0.38*
Terrigenous	<i>Al</i>	0.35*	0.54***
	<i>Fe</i>	0.36*	0.48**
Natural	<i>Co</i>	ns	ns
	<i>Ni</i>	ns	0.35*
Mixed	<i>As</i>	ns	0.38*
	<i>Cr</i>	0.33*	0.54***
	<i>Mn</i>	ns	ns
	<i>S</i>	ns	0.57***
Inorganic contaminants (EF)	<i>Ag</i>	0.41*	0.70***
	<i>Ba</i>	ns	ns
	<i>Bi</i>	0.50**	ns
	<i>Cd</i>	ns	ns
	<i>Cu</i>	0.39*	0.55***
	<i>Hg</i>	0.41*	0.51**
	<i>P</i>	ns	ns
	<i>Pb</i>	0.45**	ns
	<i>Sb</i>	ns	ns

	<i>Sn</i>	ns	ns
	<i>Ti</i>	ns	ns
	<i>Zn</i>	0.44**	0.48**
Organometallic contaminants	<i>MMHg</i>	0.38*	0.59***
	<i>TBT</i>	0.06*	0.73***
	ΣBT	0.47*	0.73***
Organic contaminants	<i>Fluoranthene</i>	ns	ns
	ΣPAH	ns	ns
	<i>CB-153</i>	ns	0.47*
	ΣPCB	ns	0.47*
Organic matter	<i>POC</i>	ns	ns
Sediment potential toxicity	<i>Metal based m-ERM-Q</i>	0.42**	0.55***
	<i>m-ERM-Q with PAHs</i>	0.50*	ns

FIGURE CAPTIONS

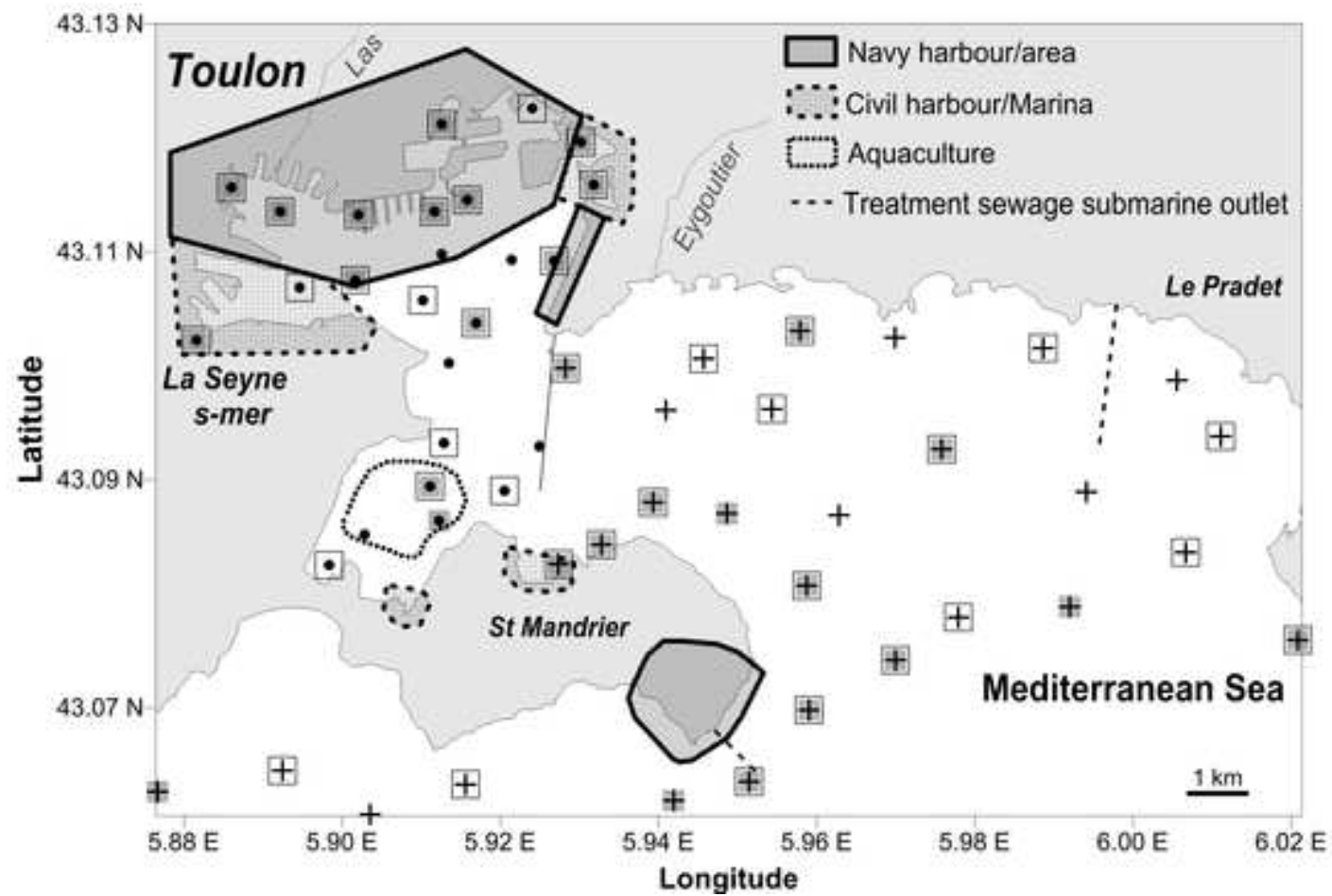
Figure 1. Sampling stations and main anthropogenic activities in Toulon bay. *Dots represents the specific sites where organic contaminants were quantified, small grey squares represent the sampling sites with butyl-tin species measurements and large open squares represent the sites from which samples were analyzed through T-RFLP.*

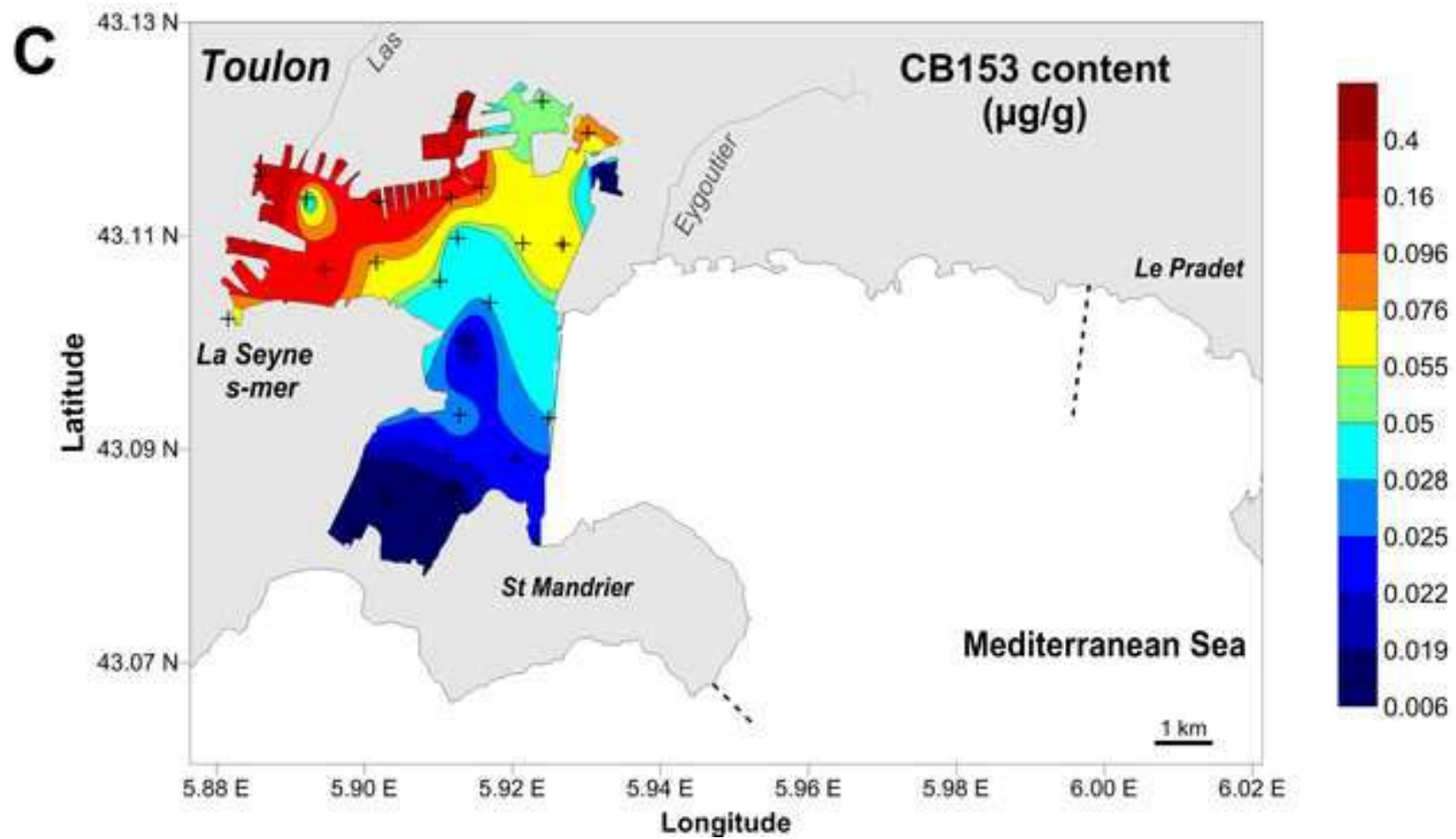
Figure 2. Examples of benthic abiotic spatial gradients in the Toulon bay. **A, B, C:** contents of Al, Cd and CB-153, respectively. **D:** Gower similarity, the white arrows represent the reference sites. **E:** *m-ERM-Q* calculated with toxic metals' concentrations. Scales were drawn according to deciles of the respective datasets.

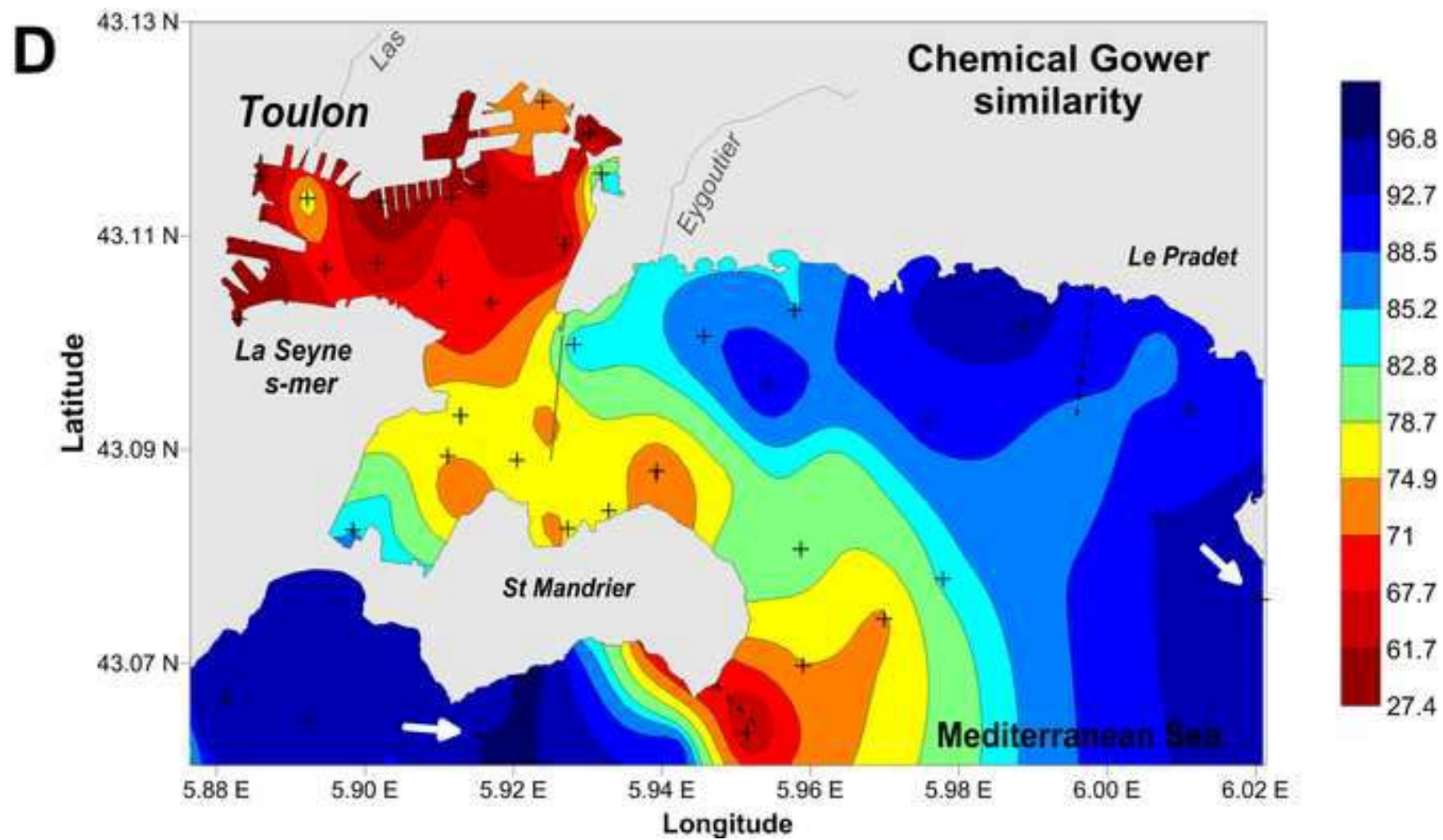
Figure 3. Spatial variations of prokaryotic richness and community structure in surface sediments of Toulon bay, calculated from t-RFLP data. **A and B:** bacterial and archaeal richnesses, respectively. **C and D:** Bray-Curtis similarities of T-RF structure within bacterial and archaeal communities, respectively. Arrows design the reference sites used for representing similarities.

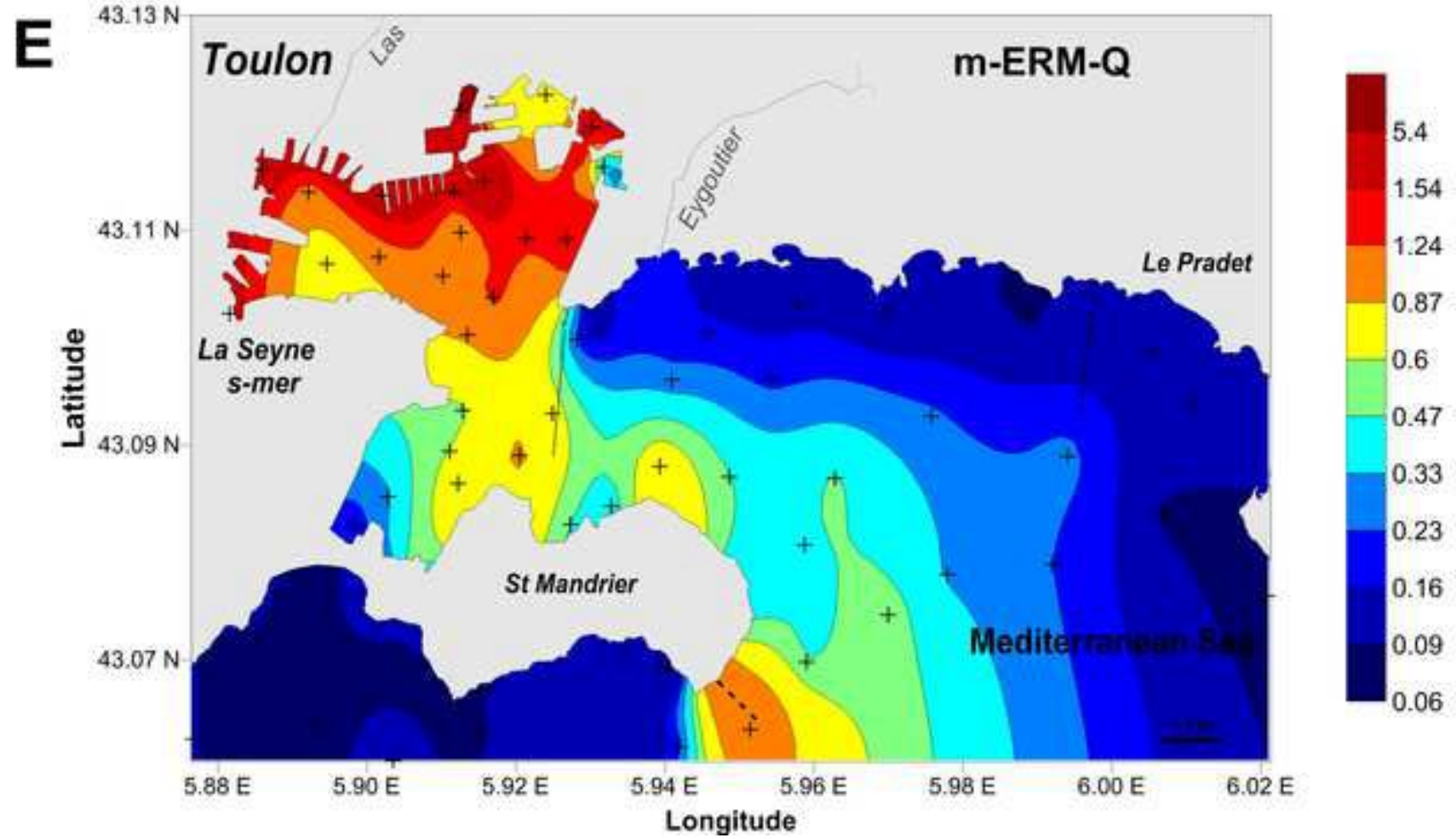
Figure 4. PCoA of bacterial (**A**) and archaeal (**B**) communities using Bray-Curtis distances, calculated from normalized tRFLP data. *LiB: Little Bay, LaB: Large Bay*

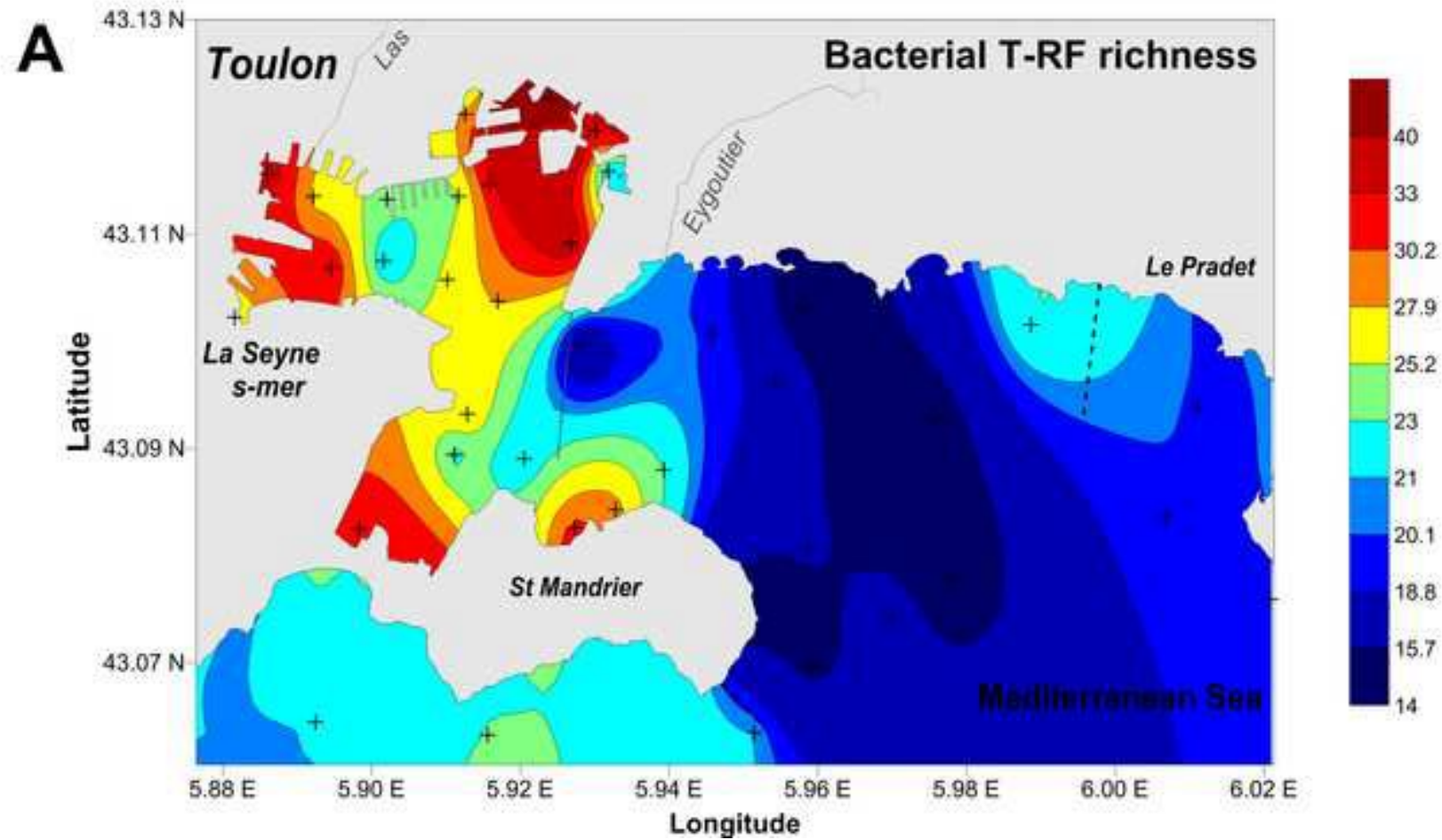
Figure1
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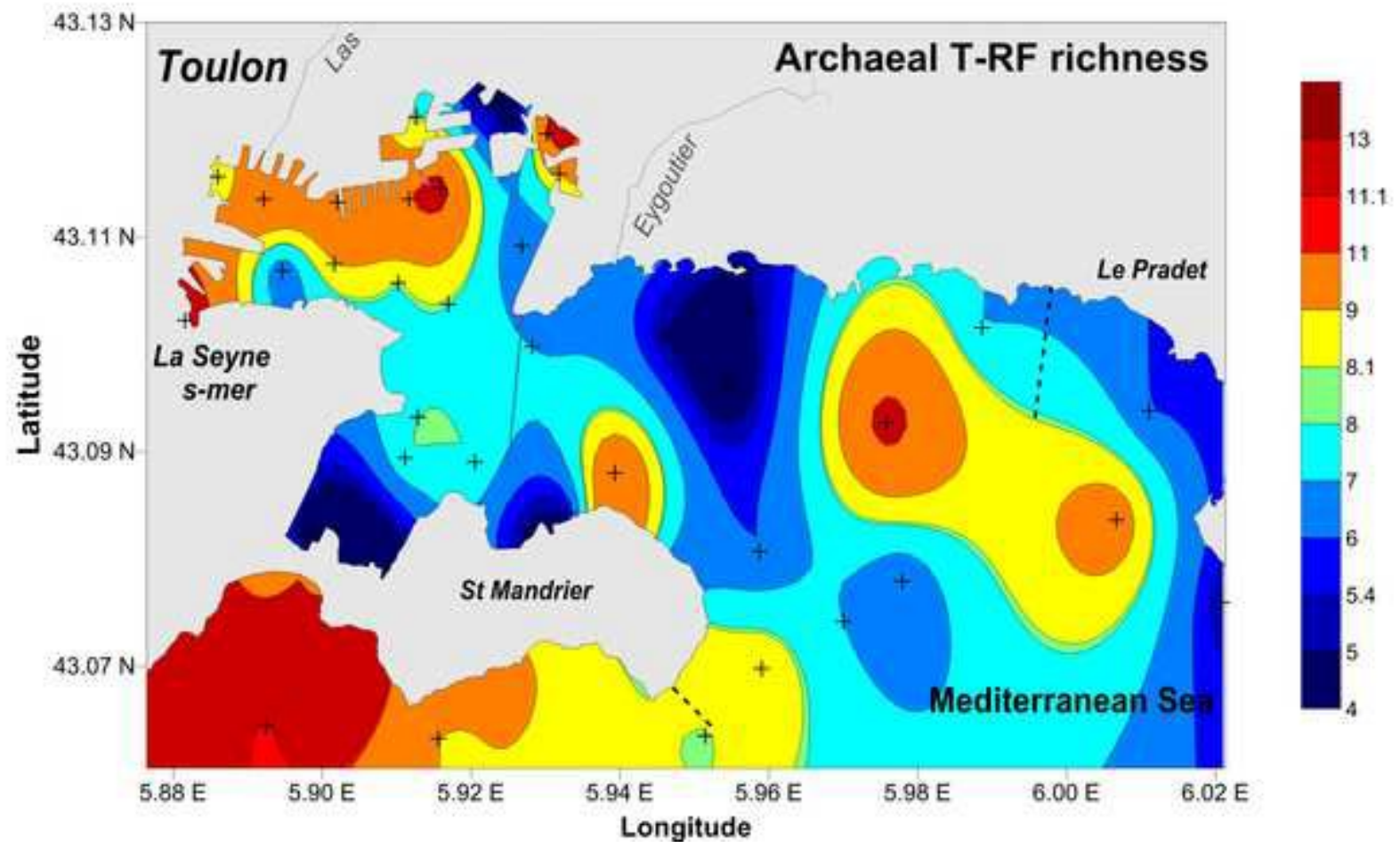




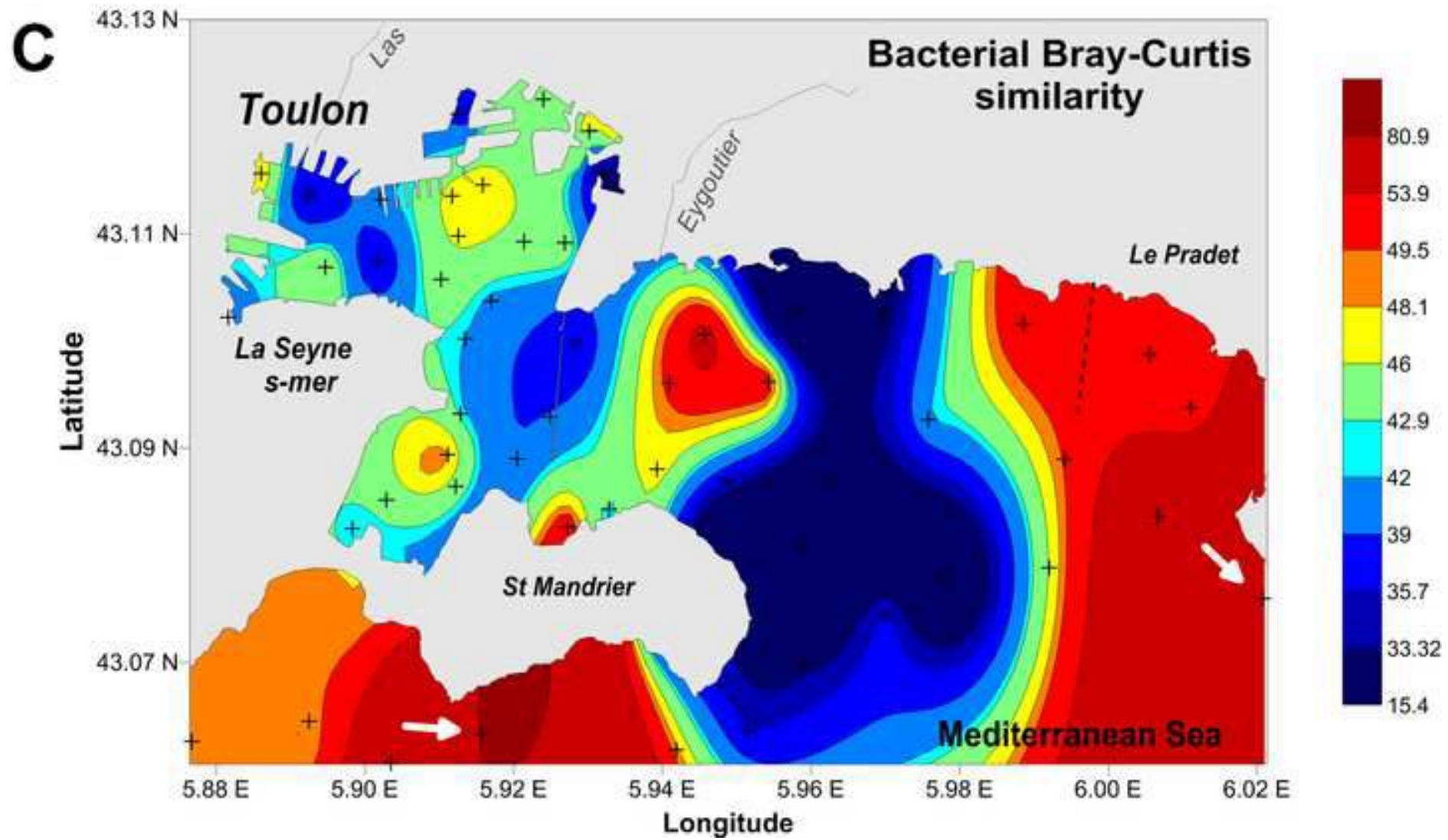


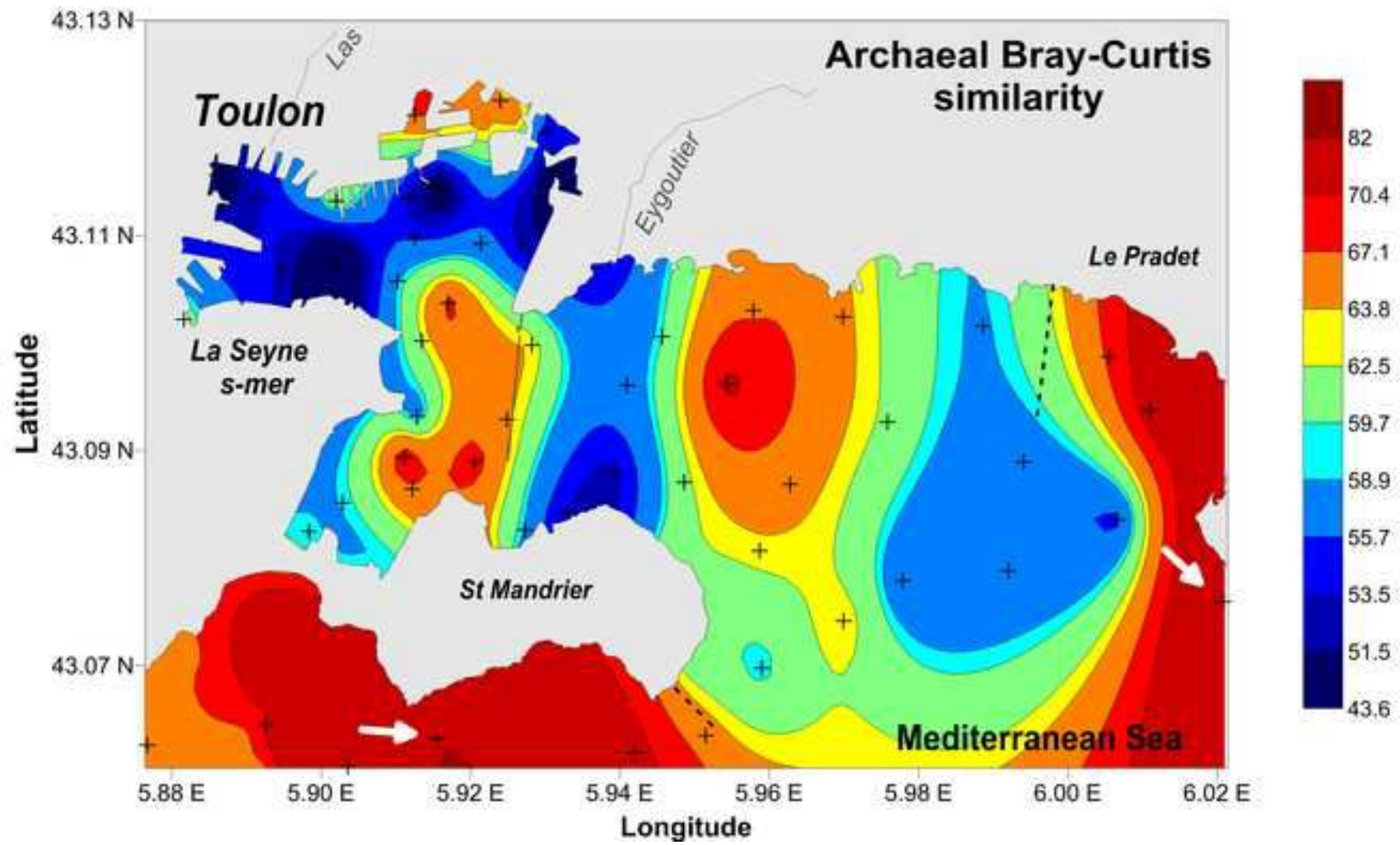




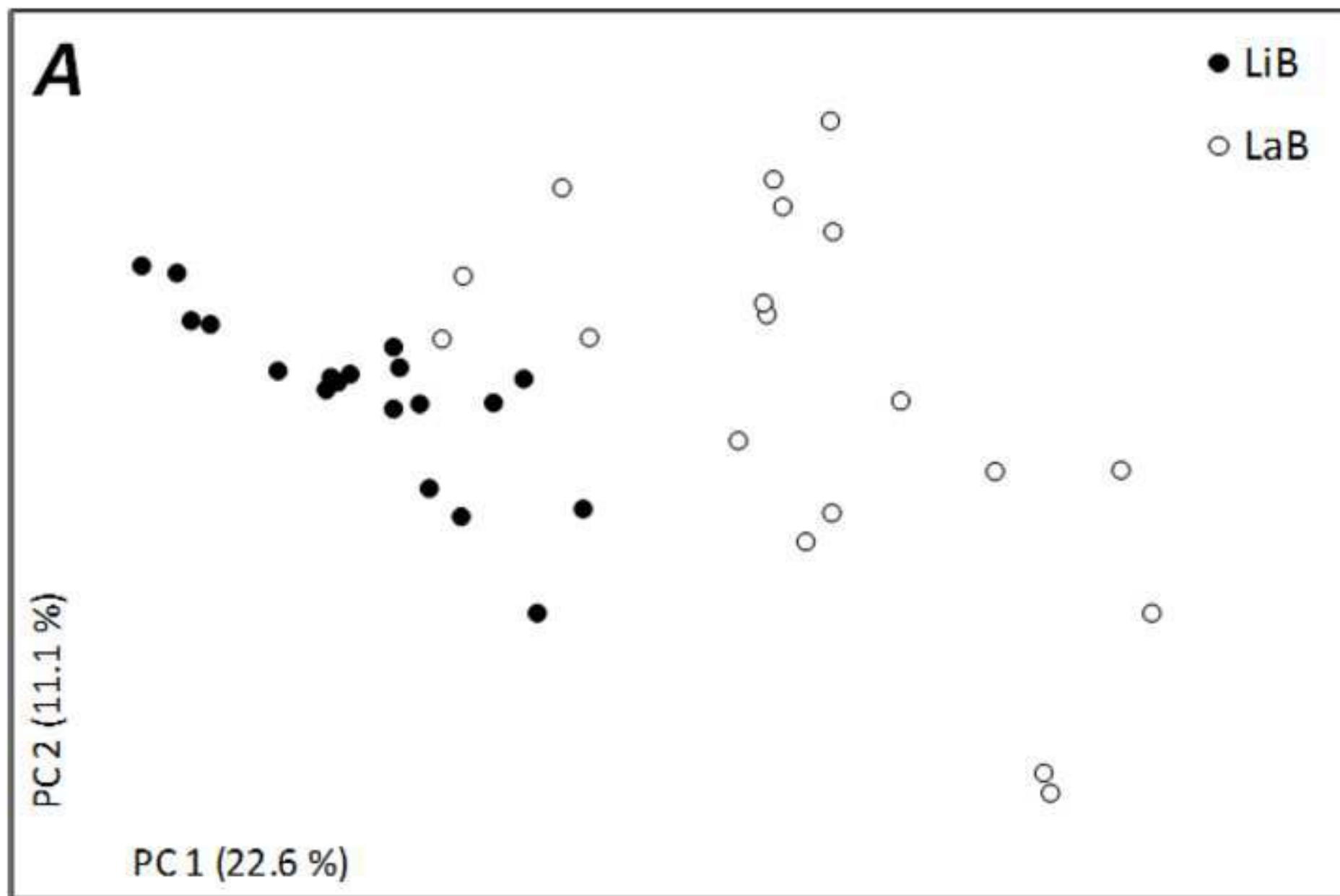


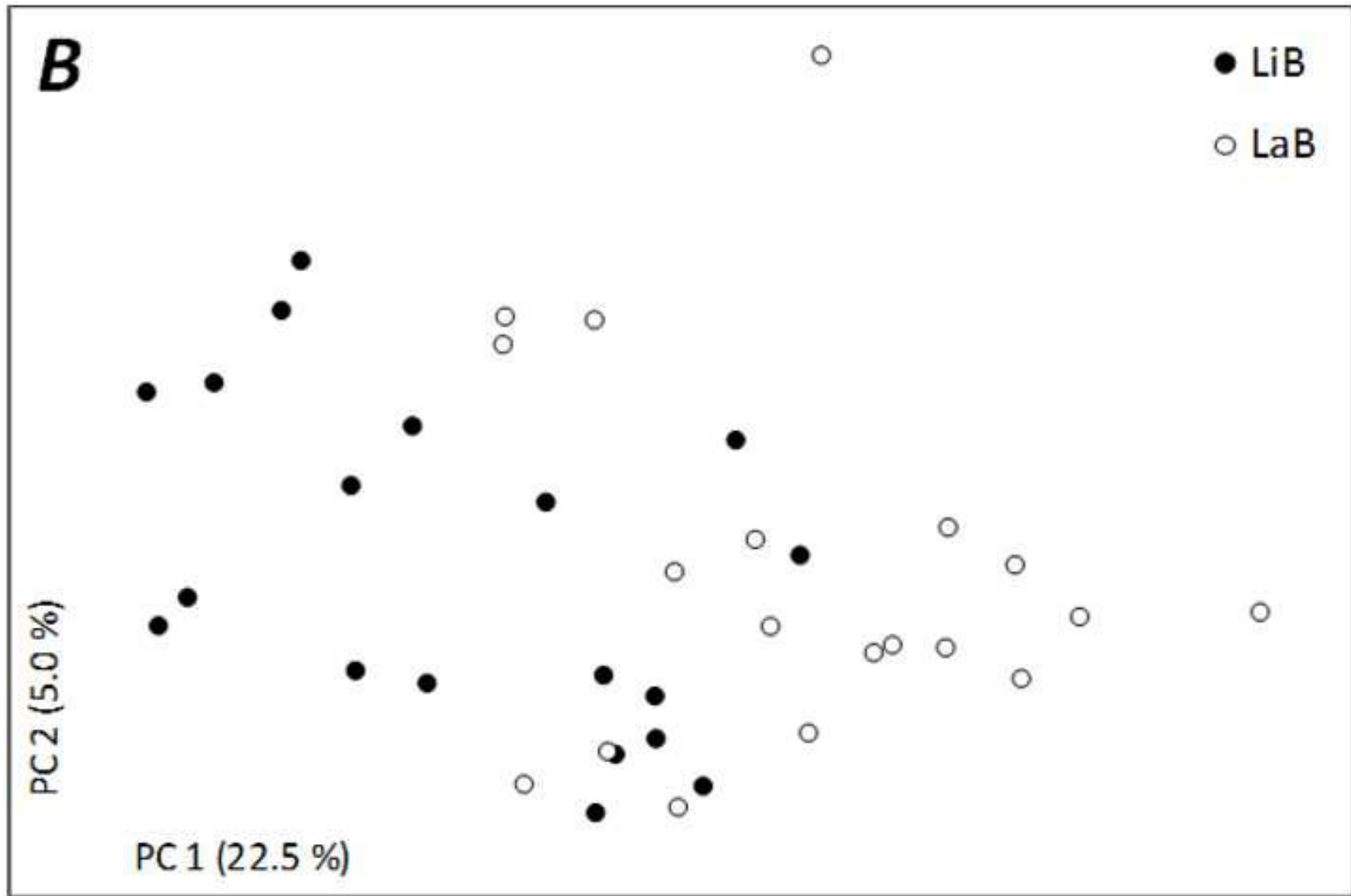
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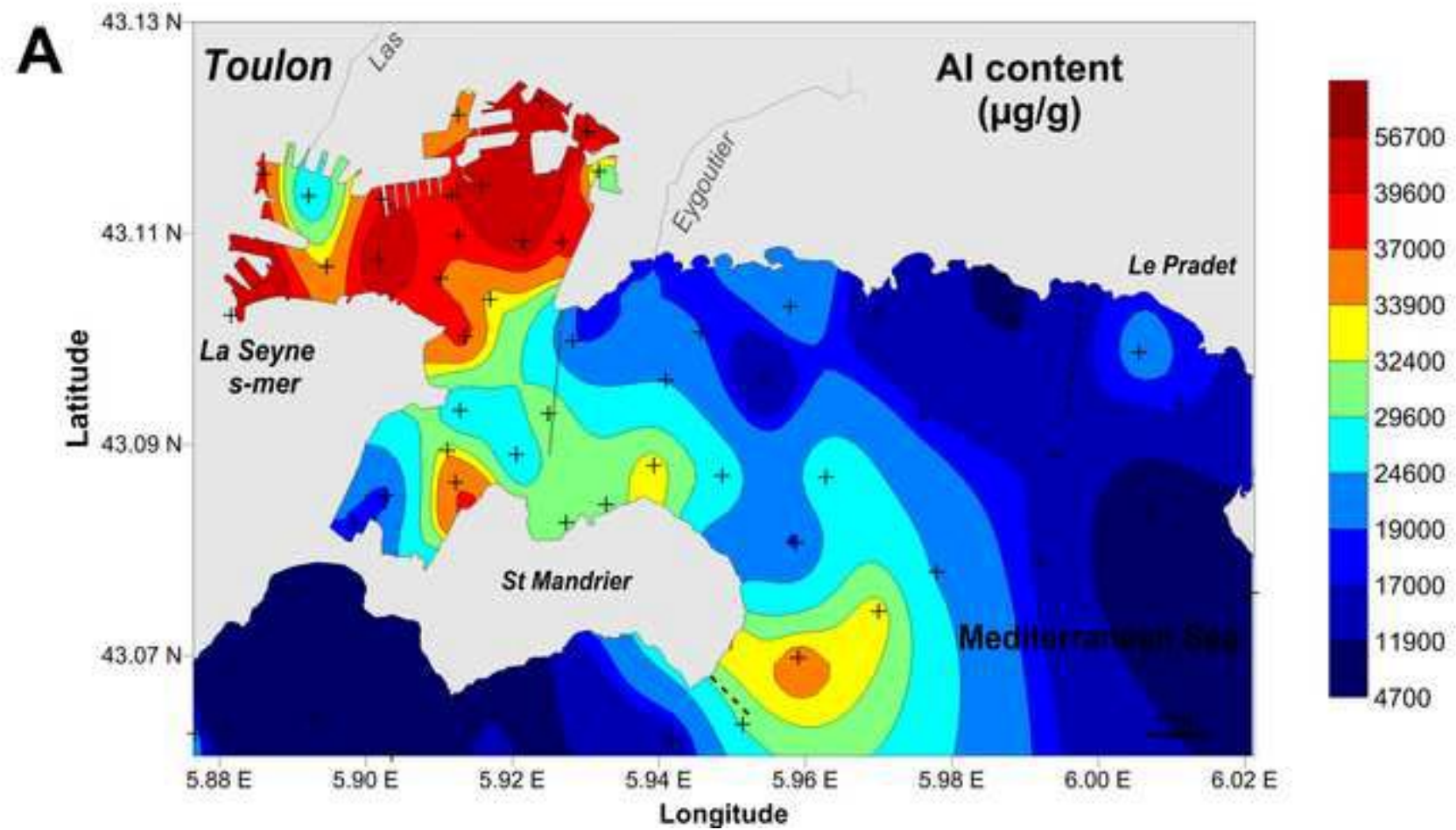


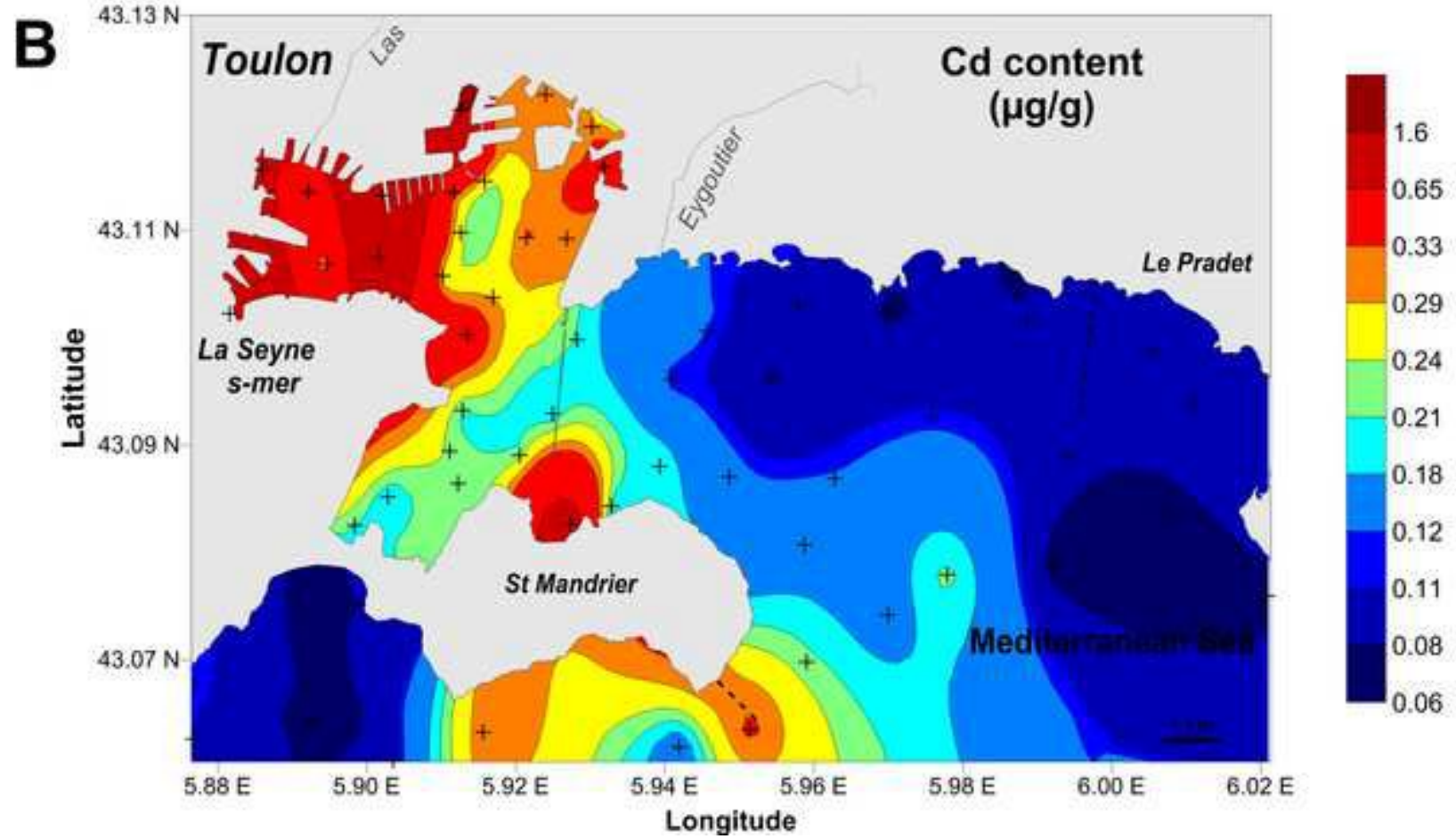


D









Supplementary material for on-line publication only

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